WATER CONTENT AND TEMPERATURE AS FACTORS INFLUENCING DIASTASE FORMATION IN THE BARLEY-GRAIN

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I INTRODUCTION

The hydrolysis of starch-paste by germinating barley was observed by Kirchoff in 1814. The first account of an enzyme in solution is that of Dubrunfaut (1830). He found that an aqueous extract of malt converted starch-paste to sugar. The active agent for this conversion was not discovered until 1833. In that year Payen and Persoz (1833) precipitated by means of alcohol, from an aqueous extract of malt, a white powder which had the power of converting starch-paste to sugar. This powder they called diastase. Since its discovery our knowledge of diastase has greatly increased. Its formation, distribution, and behavior to temperature, chemical agents, etc., have been the subject of especial investigations, and excellent discussions of the literature may be found in Green (1901), Effront (1902), Czapek (1913).

Comparatively little is known concerning the relative activity of diastase, its increase or its decrease, in the resting seed under different conditions of moisture, temperature, etc., in storage. Since the quantity of water in the seed varies considerably under different conditions of storage and relatively small variations have a marked effect on fundamental life processes, as for example, respiration, it was thought worth while to investigate the effects of moisture and temperature on the grain in storage. White (1909) in studying the latent life of resting seeds, found that active diastase is present in them in an appreciable amount. The diastase and other enzymes, "retain their activity without appreciable change in stored dry seeds for twenty or more years, that is long after the power of germination has been lost, which takes place in wheat after eleven to sixteen years, barley eight to ten years, oats five to

nine years, maize and rye over five years. The life of the stored seeds is largely dependent upon the climatic conditions, a dry climate favouring longevity."

The experiments of Kolkwitz (1901) on the respiration of barley as affected by moisture content are of interest, since it is through the hydrolization of starch by diastase that active and long continued respiration depends. Kolkwitz found that 1 Kilo. of barley with a moisture content of 10 to 11% gives off in twenty four hours from 1/3 mg. to 1 1/2 mg. of CO_2. In barley containing 20% water the respiration is markedly higher than could be attained through any increase of temperature in air-dry barley, that is, barley containing 10 to 11% water. One Kilo. of barley of 33% water content gives off in 24 hours 2000 mg. of CO_2, and this quantity of CO_2 may be increased ten fold by an increase in temperature and an increase in the quantity of oxygen supplied. Duvel (1904), Babcock (1912), and others have had similar results.

II MATERIALS AND METHODS

Water content and temperature are the two factors whose effect on the diastase content of the resting seed it was planned to study. Both factors must be controllable within narrow limits over considerable periods of time. Much time was used in working out methods by which the temperature and more especially the moisture content was maintained through longer periods of time and with relatively slight variations. Kolkwitz (1901) experienced a similar difficulty. He says, "Die Hauptschwierigkeit dieser Untersuchungen lag in der Wahl einer geeigneten Methode, denn ein Schwanken im

Feuchtigkeitgehalt der hygroskopischen Körner während des Versuches hätte die Genauigkeit der Resultate ernstlich in Frage gestellt."

Manshury barley obtained from Vaughan Seed Store, Chicago, was first used. It was given up and White Hulless substituted because it was found impossible to distinguish between the moisture held in the outer covering of the kernel and that of the kernel proper. Brown (1909) experienced a similar difficulty. He says, "It should be pointed out that the determinations on which the curves are based are affected by an unavoidable error due to the manner in which the values are arrived at. Some allowance should be made for the amount of water absorbed by the outer covering of the seeds. It is not possible to evaluate this amount very closely; apparently, however, it may be taken as equal to about 8% of the original weight of the dry seed." An error of 8% in moisture content is far too large, since the respiration, as we have already seen, is markedly affected by far smaller moisture variations. We shall see later that this is also true of diastase activity in the seed.

Moisture Control.- Two methods, both successful, were used to bring the barley grains to the desired moisture content. The first, which might be termed the immersion method, was used when a relatively high water content was desired and the period of experimentation did not exceed 12 hours. The second, which might be termed the vapor tension method, was used in the greater number of experiments. It permits of a far more delicate control of moisture content and the content once reached, can be maintained, under proper conditions, almost indefinitely. It cannot be used where a moisture content sufficiently high for germination, or nearly so, is desired.

The Immersion Method. - Brown (1909) first called attention to the fact that the membranes covering the barley grains were semipermeable in nature. In consequence of this and the strong affinity for water of the grain itself, it is possible by varying the density of the solution to furnish the grain a definite quantity of water. Schroeder (1911) and Shull (1913) have applied and extended this method.

A definite quantity of barley grains was placed directly into a saturated solution of LiCl. This, according to Shull (1913), gives the highest osmotic pressure of any known neutral salt and he found that the seeds of Xanthium were unable to take up water from a saturated solution. This means that the osmotic pressure of the salt, 965.3 atmospheres, is greater than the force of imbibition of the seeds. In solutions of less density it was possible for the seeds of Xanthium to take up water until the imbibition force of the seeds and the osmotic power of the solution were in equilibrium. Brown (1909) had found that the coats of the barley grain were semipermeable and that the seeds extracted water from solutions of H_2SO_4, $NaNO_3$, $CuSO_4$, and others. Tests showed that the coats of barley were impermeable to LiCl in solution and solutions of this salt were preferred to the H_2SO_4, etc., used by Brown. Much to my surprise the barley grain took up considerable water from a saturated solution of LiCl, thus showing it to possess an imbibition force much greater than that of Xanthium.

Twelve test tubes each with 10 c.c. of a saturated solution of LiCl, were heated in a water bath to 30°C. Into each tube 100 air-dry barley grains, whose weight had been previously determined, were placed. Each tube was stoppered with a perforated rubber

stopper with a capillary tube to allow an equilization of pressure during the heating. The tubes were then immersed in the bath, so that the upper end of the glass tube only projected above the water. An equal number of samples of barley grains were treated in a like manner except that distilled water was substituted for the salt solution. Tubes containing air-dry barley served as checks. At intervals of two hours, three tubes, one containing a sample in the solution, the second containing a sample in distilled water, and the third containing the air-dry check, were removed from the bath. The grains were superficially dried and their weight again determined. They were then placed on wire nets and dried in a current of warm air.

The Vapor Tension Method.- The vapor tension above a salt solution of definite concentration held at a constant temperature is definite and constant. It is possible by changing the temperature of a solution of given density to raise or lower the vapor tension above the solution. By changing the density of the solution and maintaining a constant temperature, it is possible to obtain a wide range in vapor tension.

Half-gallon fruit jars were used as containers in each case. The tops were supplied on the inside with a hook for suspending the wire containers described below. The jars were supplied with solutions as follows:

1. 400 c.c. saturated solution of $CaCl_2$. Twadell reading -- 75.7°
2. 200 c.c. saturated solution of $CaCl_2$ plus 200 c.c. of distilled water. Twadell reading -- 40.0°
3. 100 c.c. saturated solution of $CaCl_2$ plus 300 c.c. of distilled water. Twadell reading -- 20.5°

4. 50 c.c. saturated solution of $CaCl_2$ plus 350 c.c. of distilled water. Twadell reading -- 9.0°

5. 400 c.c. water -- Twadell reading -- 1.1°

After the jars were sealed they were placed into large temperature cases for 24 hours, before the wire containers containing the barley were placed into them. By this means, there was set up a series of jars whose moisture content varied from the lowest to the highest possible under the prevailing temperature. Five series were thus maintained at temperatures as follows: 5°C, 10°C, 15°C, 20°C, 25°C.

After the jars had remained in the cases a sufficient length of time for the solutions and the air above them to reach an equilibrium, the jars were furnished with the wire baskets containing 50 grams of barley each. The cylindrical baskets were made of wire netting of fine mesh and were suspended by a bale from the hook in the top of the jar. The lower end of these baskets was 5 centimeters above the liquid. Each container was weighed several times a day until it attained a constant weight.

As soon as a container attained a constant weight, a weighed amount of grains was removed and rapidly dried. The drying in this case took place in a drier especially constructed for the purpose. By means of a blower, heated air was driven over wire pans which were arranged in tiers and covered with a large tubulated bell-jar. Grains with the largest quantity of water used in these experiments were thoroughly dried in two hours.

When the seeds had become thoroughly dried they were ground to a fine powder. Five-tenths of a gram of this ground material was taken from each sample and digested in fifty times its weight of

distilled water for one hour at 50° C. During the process of digestion, the mixture was thoroughly stirred at frequent intervals. The extract obtained was placed in a centrifuge and rapidly rotated for ten minutes in order to remove all suspended materials, especially starch grains. This treatment was found to be necessary, for the amount of starch in the ground material was sufficient to introduce a large error in the results.

The Starch Solution.- A .2% solution of soluble starch was used in all of the determinations for diastase. This was prepared by dissolving an accurately weighed amount of Lintner's soluble starch in boiling distilled water. A fresh solution was made for each determination.

Diastase Activity.- The diastase activity (Abderhalden 1910) (Oppenheimer 1911) of the ground barley was determined by adding 4 c.c. of the diastase solution to 20 c.c. of the starch solution. The mixture was placed in a 50 c.c. flask which was then immersed in the bath until only a small portion of the neck extended above the water. At intervals, 2 c.c. of this solution were removed from the flask and added to an equal quantity of distilled water in a test-tube. It was rapidly brought to boiling and allowed to cool to room temperature. Each tube was then tested with three drops of a .1 normal iodine solution, which had been standardized by titrating it against a solution of sodium thio-sulphate. The tube which gave no test for starch or any of the dextrins when the iodine was added, was selected as the one determining the end point of the conversion. The moisture content of the air-dry barley was ascertained and the diastase activity determined in a like manner.

Temperature Control.- In the course of experimentation, two

different temperature controlling devices, a water bath with thermostatic control and a temperature ease, were used. The former was used for experiments at 30°C. For lower temperatures than 30°C. this was impracticable and the cases with chilling devices were employed.

The bath consisted of a circular tank encased in an asbestos covering and had a capacity of about 30 gallons. The 'two-heat' heating unit which was used, was controlled by an immersion thermostat. The temperature regulation was accomplished by the cutting on and off of the high heat which allowed the low heat to be constantly on. To maintain the bath to within a variation of .5°C. it was necessary to use a cooling device in order to prevent a rise in temperature after the high heat had been cut off. Satisfactory results were obtained by passing a constant stream of cold water through a pipe which was placed horizontally in the middle of the bath. In place of the stirring methods which have been previously used, the water was kept constantly in motion by passing bubbles of air under pressure through it. This arrangement gave better results than any of the previous ones which were tried and had the added advantage of requiring practically no attention.

In order to keep the starch solution at an even temperature during its conversion by the diastase extract, another bath was set up and maintained at a temperature of 50°C. Because of the high temperature of the bath the evaporation was very fast. This evaporated water was replaced by a constant level apparatus.

III EXPERIMENTAL RESULTS AND DISCUSSION

The air dry barley grain under the storage conditions of the laboratory contained 8.76 percent of water. At temperatures of 5, 10, 15, 20 and 25°C., the diastase of this grain as determined by the period required to hydrolize a starch solution of given concentration is 44 hours 00 minutes, 44 hours 00 minutes, 42 hours 45 minutes, 42 hours 15 minutes, and 41 hours 15 minutes, respectively. These results, Table I and Graph I, express the temperature effect on diastase in barley with 8.76 percent of water. The quantity of diastase in the barley grain at the above temperatures varied only slightly during a period of from two to three weeks. At a temperature of 30°C the initial diastase is slightly increased and this continued for the period of exposure - 12 hours. The results are shown in Table II.

The results given in Table IIIb show quite clearly that the vapor tension method is well adapted for furnishing different amounts of water to samples of air dry grain. At 5°C. the water content of barley grains suspended above solutions of different concentration from distilled water to a saturated solution of $CaCl_2$, varied from 10.91 to 32.48 percent. Above a saturated solution of $CaCl_2$, air dry barley grains take up only 2.15 percent of water but when placed above a solution approximately half as dense, (1-1), they absorb 7.68 percent,. or about 3.5 times the quantity. When the density of the solution is again reduced by mixing one part of a saturated solution of $CaCl_2$ with three parts of distilled water, (1-3), the amount of water taken up by the grains is almost double that taken up by grains placed above a solution of equal parts of distilled water and a saturated solution of $CaCl_2$. A further decrease in the density, (1-7), causes another increase in imbibition but in this instance it is only

2 percent more than in the last mentioned case. Grains suspended above distilled water show a concomitant increase in the amount of water taken up. In this case it is about 2 percent higher than in grains held above the greatest dilution of $CaCl_2$ used. The above method enables us to study the diastase of the barley grain at 5° C. with a water content of 10.91 percent, 16.44 percent, 22.34 percent, 24.72 percent, 26.83 percent respectively.

At 10° C., above solutions of $CaCl_2$ of the same concentrations as used at 5° C., the grains again give us an ascending series in respect to the water content and differ from the first (5° C.) in that the content above the respective solutions is slightly greater. For example, the increase above the saturated solution of $CaCl_2$ is .11 percent, that above the solutions 1-1, 1-3, 1-7, and distilled water is 1.1, 1.34, 1.04, and 3.08 respectively. The amount of water taken up by barley grains at 15° C., when suspended above the different solutions, increases as the density decreases but the increase does not take place as uniformly as at the previous temperatures. These irregularities are similar to those observed by VanBemmelen (1910). They will receive further attention in a later article.

The amount of water taken up by the grains at 20° C, above the series of solutions, again assumes a regular increase as the density is decreased. The amount of water taken up above distilled water is about eight times as great as that absorbed above a saturated solution of $CaCl_2$. The same progressive increase in the amounts of water taken up by the grains is also found when the solutions are maintained at a temperature of 25° C.. In this case the amount of water which the grains take up when suspended above distilled water is about seven times greater than that absorbed above a saturated

solution of $CaCl_2$.

The rate of hydrolysis as shown in Table IV shows that the method allows a study of the effects of temperature and water content on the diastase in the dormant barley grain. The factors, temperature and water content, have been the repeated subjects of investigation on the respiration of barley grains. The results show us a general uniformity in that a rise of temperature as well as a rise in water content markedly affects the respiration. As already stated, the experiments of Kolkwitz (1901) first acquainted us with the effect of increased water content of the barley grain on its respiratory activity. Other investigators have had similar results. Bailey and Gurjar (1918) have recently contributed an elaborate investigation on the relation between respiratory activity and water content of stored grain. Their results on the respiration of Haynes Bluestem (Minnesota 169) wheat is of especial interest and is reproduced below.

Moisture	Carbon dioxide respired per 24 hours for each 100 gm. of dry matter	Moisture	Carbon dioxide respired per 24 hours for each 100 gm. of dry matter
Percent	Mgm.	Percent	Mgm.
12.50	0.54	16.08	2.88
13.93	.65	16.65	6.86
14.78	.86	17.07	11.72
15.42	1.62		

The water content of the air dry grains increased only slightly when suspended above a saturated solution of $CaCl_2$ at 5, 10, 15, 20 and 25°C. respectively. The total increase of water content in the range of temperatures used is 1.07 percent. Above a solution of

half that density, an increase in temperature of 5°C. is sufficient to cause an increase in water content (1.07 percent) equivalent to that obtained throughout the whole range of temperature (5°C. to 25°C.), above a saturated solution. In the range of temperatures used (5°C. to 25°C.), an increase of 4 percent in the water content was observed. Above the other concentrations of the salt solutions an increase of 5°C. produces an increase of from 1 percent to 1.5 percent in the amount of water imbibed. When distilled water is used, the effect of the temperature is about the same as in the more dilute salt solutions; an increase of 5°C. producing an increase of from 1 percent to 1.5 percent in the water absorbed.

In column 5 of Table Vb are given the amounts imbibed, in percent, by barley grains immersed in a saturated solution of LiCl at 30°C. In this case it is the time factor and the approach to imbibitional equilibrium that produces the differences in water content. At the end of two hours the grains absorb 14.85 percent of water, that is, have a total water content of 23.61 percent - water content of air dry grains, 8.76 percent plus 14.85 percent increase. The quantity of water taken up was unexpectedly large. The results obtained by Shull in his experiments with Xanthium show a much lower imbibition-force. The results obtained with barley indicate that the imbibitional force exerted by the barley grain, expressed in osmotic pressure, is far in excess of that of the seed of Xanthium. At the end of four hours the grains had taken up 19.07 percent of water, an increase of 5.22 percent. Each two-hour increase in the time of immersion produces an increase in the amount of water taken up, but the increase gradually lessens as the imbibitional forces reach more nearly equilibrium. The gain of 5.22 percent of the two-hour period

is not again reached.

The amount of water, expressed in percent, taken up by barley grains immersed in distilled water at 30° C. is given in column 5 of Table VI. At the end of two hours the grains had taken up 21.27 percent of water, an amount 1.3 times greater than that absorbed from a saturated solution of LiCl in the same length of time. At the end of four hours 29.17 percent of water had been absorbed, an amount 1.2 times greater than that at the end of two hours. Here again as the grains are immersed for a longer time the amount of increase is successively less until at the end of twelve hours (55.01 percent) it is only 3.53 percent more than at the end of ten hours (51.48 percent). The decrease is not so rapid as in the case of a saturated solution of LiCl. Upon comparing the water content of the barley grains after having been immersed for twelve hours in a saturated solution of LiCl and distilled water respectively, it can be seen that they are to each other as 1:1.26. This method of moisture control permits the grains to take up a much larger quantity of water than was possible with the vapor tension method. The quantity of water taken up by the grains from a saturated solution of LiCl in two hours time is over four times that taken up when suspended above a saturated solution of $CaCl_2$ maintained at a temperature of 25° C.

The results, Tables VII, VIII, IX, X and XI, Graphs II, III, IV, V and VI, clearly show that diastase formation in the barley grain is markedly affected by water content and by temperature.

At 5° C., a temperature a little above the minimum for diastase activity, an increase in the water content of the grains shows a rather marked effect on the diastase. At this temperature grains containing 10.91 percent of water show a diastase activity of

approximately 37, as indicated by the hours required to convert the starch in 20 c.c. of a .2 percent solution. With a water content of 16.44 percent the diastase activity is 34 1/2. Thus, (Table III a) the water content of grains above a saturated solution of $CaCl_2$ (5.45 grams) and that above a solution, 1-1, (8.22 grams), are as 5.45:8.22. The diastase of the same samples is as 36.8:34.3, expressed in the period of time required to hydrolize the starch solution. Likewise the water content of grains above a solution, 1-3, (11.17 grams), and that above a solution, 1-1, (8.22 grams), are as 11.17:8.22, while the diastase from the same samples is as 29.8:34.3. In view of these facts and since a constant temperature was always maintained, it is evident that the water content of the grains is an important factor influencing diastase formation.

Similar comparisons may be drawn between the water content and the diastase formation at the other temperatures used. For example, at 10°C. as shown in Table VIII, the grains above a saturated solution of $CaCl_2$ contained 11.02 percent of water while those above distilled water contained 29.91 percent, a difference of about 19 percent. The water content (Table IIIa) in the first sample (5.51 grams) is to the water content in the second sample (14.95 grams) as 5.51:14.95, while the diastase in the two samples is as 36.25:24.25. While the percent of water absorbed by the grains above the various solutions at 15°C. do not seem to conform to the amount absorbed at the other temperatures, from the results given in Table IX, the diastase formation still seems to conform to the amount of water in the grains. Thus, while the grains contained more water when suspended over a solution, 1-7, than they did when held above distilled water, the time for conversion in the first case is about thirty minutes less than in the latter. In other words, the water content (Table

IIIa) of the first sample (15.35 grams) is to the water content of the second sample (14.37) as 15.35:14.37. The diastase in these samples is as 25.5:25.8. Likewise, Table X shows that at 20°C. the diastase formation is dependent on the water content of the grains since in the samples an increase in the water content of from 11.51 percent to 30.97 percent shows a concomitant decrease in the conversion time; that is, an increase in diastase formation. The water content of the grains suspended above a saturated solution (5.75 grams) is to the water content of the grains suspended above distilled water (15.48 grams) as 5.75:15.48. The diastase of the same samples is as 35.5:23.1. This is also true of samples maintained at a temperature of 25°C. (Tables XI and IIIa). In this instance the water content of the grains above a saturated solution of $CaCl_2$ (5.99 grams) is to the water content of the grains suspended above distilled water (16.24 grams) as 5.99:16.24. The diastase of these same samples is as 34.8:20.

Tables V and VI give a comparison of the water content and of the diastase activity of grains at 30°C. Here, as in the previous experiments, the dependence of the diastase formation on the water content is clearly shown, as each increase in water content causes a concomitant decrease in the time necessary for conversion. Thus, the water content (Table Va) of the grains after two hours (.85 gram) is to the water content after twelve hours (1.37 grams) as .85:1.37. The diastase of the same samples is as 7.6:4.75. There is also a marked difference between the diastase of the samples which have been immersed in a saturated solution of LiCl and distilled water respectively, since the quantity of water in the former is to the latter as 1:1.15.

While the water content at a given temperature markedly affects the diastase formation, it must be borne in mind that an increase in temperature toward the optimum of diastase formation likewise produces an effect. A closer study of the figures in Tables IIIa, IIIb, and IV, shows that a difference of .17 percent in the amount of water contained in the samples of grains suspended over the solution, 1-3, at temperatures of 15° C. and 25° C. respectively, causes a reduction of the time required for conversion about 1 1/2 hours at the latter temperature. In other words, the water content (12.45 grams), Table IIIa, at 15° C. is to the water content (12.53 grams) at 25° C. as 12.45:12.53. The diastase from the same samples is as 28:26.5. Likewise the water content (15.29 grams) above a solution, 1-7, at 25° C., is to the water content (14.95 grams) above distilled water at 10° C. as 15.29:14.95. The diastase from the same samples is as 23.75:24.25. Although the quantity of water contained in the sample of grains above distilled water at 5° C. and that contained in a sample above a solution, 1-3, at 25° C., is rather wide, the time required for conversion in both cases is approximately the same. That is, the water content (13.41 grams) in the first sample is to the water content (12.53 grams) in the second sample as 13.41:12.53, while the diastase from the same two samples is as 26.1:26.5. The results tend to show that temperature does have an effect on the diastase formation.

The samples of barley used in these experiments were random samples. All unsound or broken grains, however, were eliminated.

IV CONCLUSIONS

1. Absorption of water by the barley grains is dependent on the temperature.

2. The coats of the barley grain are impervious to LiCl for the period of experimentation.

3. The rate of absorption of water from a saturated solution of LiCl at two-hour intervals decreases much more rapidly than from distilled water.

4. The imbibitional force exerted by the barley grain, expressed in osmotic pressure, is much greater than that of the Xanthium seed.

5. The water content of barley grains after twelve hours immersion at 30°C. in a saturated solution of LiCl and distilled water respectively is as 1:1.8.

6. When suspended over solutions varying from a saturated solution of $CaCl_2$ to distilled water, the amount of water absorbed increases in decreasing amounts, if the temperature remains constant.

7. At a constant temperature diastase formation increases with the water content of the grains.

8. Diastase formation in the barley grain is affected by temperature though not to so great an extent as by the moisture content of the grains.

9. After twelve hours at 30°C. the diastase of barley grains in a saturated solution of LiCl and in distilled water respectively is as 1:2.17.

I take pleasure at this time in acknowledging my indebtedness to Professor Charles F. Hottes, for the invaluable assistance he has given, both in the construction of apparatus and in the experimental procedure.

TABLE I

Diastatic Activity of Air-Dry Barley Grains (checks)

Temperature	Weight of grains used	Water content of grains	Time for conversion of starch solution Hrs min
5°C.	1.9383	8.76	44 - 00
10°C.	1.3815	8.76	44 - 00
15°C.	1.8657	8.76	42 - 45
20°C.	1.9138	8.76	42 - 15
25°C.	1.8614	8.76	41 - 15

TABLE II

Diastatic Activity of Air-Dry Barley Grains (checks) at 30°C.

Time in bath Hours	Weight of grains used	Water content of grains	Time for conversion of starch solution Hrs min
2	3.7211	8.76	43 - 30
4	3.8348	8.76	42 - 45
6	3.6263	8.76	41 - 45
8	3.9076	8.76	41 - 15
10	3.9549	8.76	40 - 30
12	3.7246	8.76	40 - 00

TABLE IIIa

Quantity of Water Present in Grain Used for Diastase Determination					
Solution	5° C. grams	10° C. grams	15° C. grams	20° C. grams	25° C. grams
Saturated solution of $CaCl_2$	5.45	5.51	5.25	5.75	5.99
200 c.c. sat. sol. $CaCl_2$ plus 200 c.c. distilled water	8.22	8.77	8.68	9.72	10.19
100 c.c. sat. sol. $CaCl_2$ plus 300 c.c. distilled water	11.17	11.85	12.45	12.19	12.53
50 c.c. sat. sol. $CaCl_2$ plus 350 c.c. distilled water	12.36	12.88	15.35	14.53	15.29
Distilled water	13.41	14.95	14.37	15.48	16.24

TABLE IIIb

Percent of Water in Samples Used for Diastase Determination					
Solution	5° C.	10° C.	15° C.	20° C.	25° C.
Saturated solution of $CaCl_2$	10.91	11.02	10.51	11.51	11.98
200 c.c. sat. sol. $CaCl_2$ plus 200 c.c. distilled water	16.44	17.54	17.37	19.44	20.38
100 c.c. sat. sol. $CaCl_2$ plus 300 c.c. distilled water	22.34	23.68	24.90	24.38	25.07
50 c.c. sat. sol. $CaCl_2$ plus 350 c.c. distilled water	24.72	25.76	30.70	29.06	30.58
Distilled water	26.83	29.91	28.74	30.97	32.48

TABLE IV

Determination of Diastase Activity

Average of 2 trials

Solution	5° C.	10° C.	15° C.	20° C.	25° C.
	Hrs. min.	Hrs. min	Hrs. min	Hrs. min.	Hrs. min.
Saturated Solution of $CaCl_2$	36 - 52	36 - 15	36 - 00	35 - 37	34 - 52
200 c.c. sat. sol. $CaCl_2$ plus 200 c.c. distilled water	34 - 22	33 - 15	33 - 22	32 - 22	31 - 37
100 c.c. sat. sol. $CaCl_2$ plus 300 c.c. distilled water	29 - 52	27 - 30	28 - 00	27 - 45	26 - 27
50 c.c. sat. sol. $CaCl_2$ plus 350 c.c. distilled water	27 - 30	26 - 37	25 - 37	24 - 52	23 - 45
Distilled water	26 - 07	24 - 15	25 - 52	23 - 07	20 - 00

TABLE Va

Quantity of Water Present at 30°C. in Grain Used for Diastase Determination

Time	Saturated Solution of LiCl	Distilled water
(Hours)	(Grams)	(Grams)
2	.8531	1.1984
4	1.0657	1.4667
6	1.1435	1.6512
8	1.2364	1.8463
10	1.3168	2.0638
12	1.3791	2.2452

TABLE Vb

Comparison of the Water Content of Grains Immersed in a Saturated Solution of LiCl at 30°C. with Diastase Activity

Time in solution	Weight before immersion	Weight after immersion	Gain in grams	% Gain	Time for conversion of starch solution
Hours					Hrs min
2	3.6127	4.1494	.5367	23.61	7 - 40
4	3.8323	4.5624	.7301	27.76	6 - 50
6	3.8154	4.6247	.8093	29.97	6 - 15
8	3.7905	4.6949	.9044	32.62	5 - 40
10	3.8152	4.7978	.9826	34.51	5 - 05
12	3.8732	4.9131	1.0399	35.60	4 - 45

TABLE VI

Comparison of the Water Content of Grains Immersed in Distilled Water at 30°C. With Diastase Activity

Time in solution	Weight before immersion	Weight after immersion	Gain in grams	% Gain	Time for conversion of starch solution
Hours					Hrs. min.
2	3.9897	4.8387	.8490	30.03	6 - 45
4	3.8667	4.9947	1.1280	37.93	5 - 50
6	3.8447	5.1592	1.3145	42.94	4 - 50
8	3.8676	5.3742	1.5066	47.71	4 - 05
10	4.0085	5.7212	1.7127	51.48	3 - 15
12	4.0815	5.9692	1.8877	55.01	2 - 30

TABLE VII

Solution	Comparison of Water Content and Diastase Activity at 5°C.			
	Percent of water	Diastase activity		
		1st trial Hrs. min.	2nd trial Hrs. min.	Average Hrs. min.
Saturated solution of $CaCl_2$	10.91	36 - 30	37 - 15	36 - 52
200 c.c. sat. sol. $CaCl_2$ plus 200 c.c. distilled water	16.44	34 - 45	34 - 00	34 - 22
100 c.c. sat. sol. $CaCl_2$ plus 300 c.c. distilled water	23.34	29 - 15	30 - 30	29 - 52
50 c.c. sat. sol. $CaCl_2$ plus 350 c.c. distilled water	24.72	27 - 15	27 - 45	27 - 30
Distilled water	26.83	26 - 30	25 - 45	26 - 07

TABLE VIII

Solution	Comparison of Water Content and Diastase Actvitiy at 10°C.			
	Percent of water	Diastase Activity		
		1st trial	2nd trial	Average
		Hrs. min.	Hrs. min.	Hrs. min.
Saturated solution of $CaCl_2$	11.02	36 - 30	36 - 00	36 - 15
200 c.c. sat. sol. $CaCl_2$ plus 200 c.c. distilled water	17.54	33 - 15	33 - 15	33 - 15
100 c.c. sat. sol. $CaCl_2$ plus 300 c.c. distilled water	23.68	28 - 00	28 - 15	28 - 07
50 c.c. sat. sol. $CaCl_2$ plus 350 c.c. distilled water	25.76	26 - 30	26 - 45	26 - 37
Distilled water	29.91	24 - 15	24 - 15	34 - 15

TABLE IX

Solution	Comparison of Water Content and Diastase Activity at 15° C.			
	Percent of water	Diastase Activity		
		1st trial	2nd trial	Average
		Hrs. min.	Hrs. min.	Hrs. min.
Saturated solution of $CaCl_2$	10.51	36 - 00	36 - 00	36 - 00
200 c.c. sat. sol. $CaCl_2$ plus 200 c.c. distilled water	17.37	33 - 00	33 - 45	33 - 22
100 c.c. sat. sol. $CaCl_2$ plus 300 c.c. distilled water	24.90	28 - 15	27 - 45	28 - 00
50 c.c. sat. sol. $CaCl_2$ plus 350 c.c. distilled water	30.70	26 - 00	25 - 15	25 - 37
Distilled water	28.74	25 - 30	26 - 15	25 - 52

TABLE X

Solution	Comparison of Water Content and Diastase Activity at 20° C.			
	Percent of water	Diastase activity		
		1st trial	2nd trial	Average
		Hrs. min.	Hrs. min.	Hrs. min.
Saturated solution of $CaCl_2$	11.51	35 - 15	36 - 00	35 - 37
200 c.c. sat. sol. $CaCl_2$ plus 200 c.c. distilled water	19.44	31 - 45	33 - 00	32 - 22
100 c.c. sat. sol. $CaCl_2$ plus 300 c.c. distilled water	24.38	27 - 00	28 - 30	27 - 45
50 c.c. sat. sol. $CaCl_2$ plus 350 c.c. distilled water	29.06	24 - 30	25 - 15	24 - 52
Distilled water	30.97	22 - 30	23 - 45	23 - 07

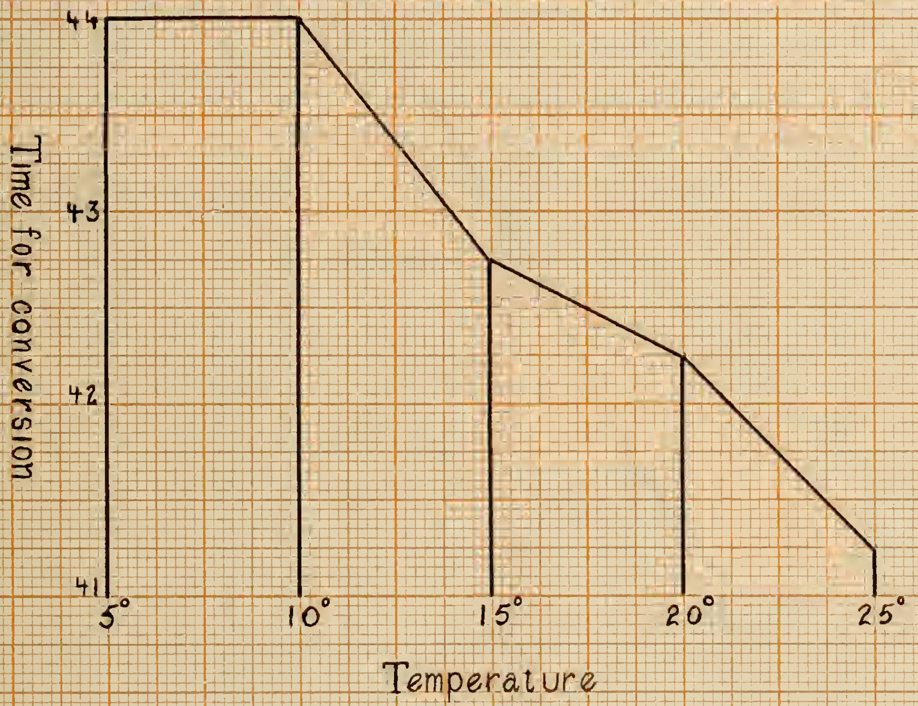
TABLE XI

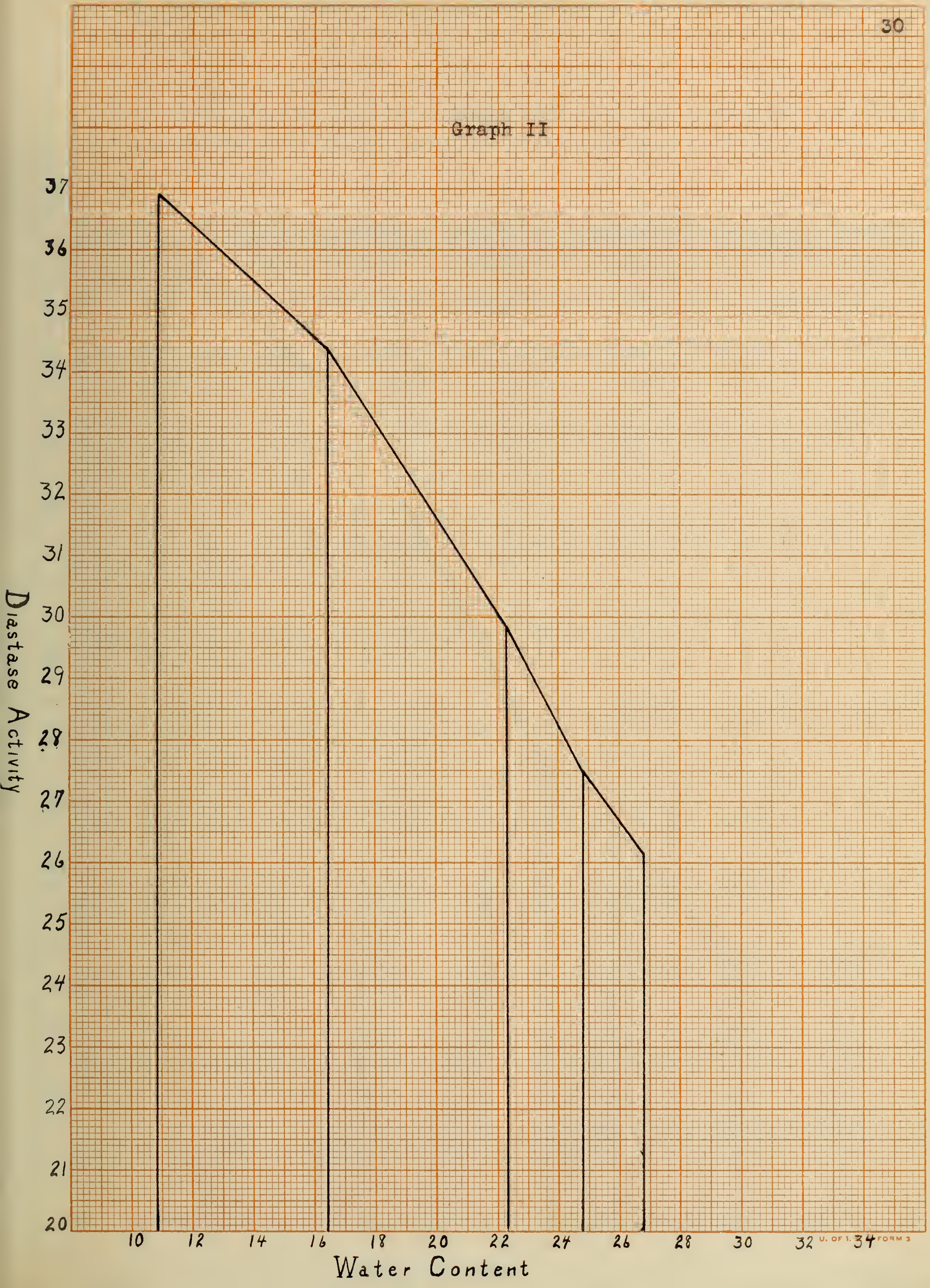
Solution	Comparison of Water Content and Diastase Activity at 25°C.			
	Percent of water	Diastase Activity		
		1st trial	2nd trial	Average
		Hrs. min.	Hrs. min.	Hrs. min.
Saturated solution of $CaCl_2$	11.98	34 - 15	35 - 30	34 - 52
200 c.c. sat. sol. $CaCl_2$ plus 200 c.c. distilled water	20.38	32 - 15	31 - 00	31 - 37
100 c.c. sat. sol. $CaCl_2$ plus 300 c.c. distilled water	25.07	27 - 00	26 - 15	26 - 27
50 c.c. sat. sol. $CaCl_2$ plus 350 c.c. distilled water	30.58	24 - 00	23 - 30	23 - 45
Distilled water	32.48	19 - 45	20 - 15	20 - 00

EXPLANATION OF GRAPHS

Graph I.	Curve showing diastase activity in the checks at 5°C., 10°C., 15°C., 20°C., and 25°C.
Graph II.	Curve showing the relation between diastase formation and water content of the grain at 5°C.
Graph III.	Curve showing the relation between diastase formation and water content of the grain at 10°C.
Graph IV.	Curve showing the relation between diastase formation and water content of the grain at 15°C.
Graph V.	Curve showing the relation between diastase formation and water content of the grain at 20°C.
Graph VI.	Curve showing the relation between diastase formation and water content of the grain at 25°C.

Graph I





Graph II

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Water Content
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Graph III


Diastase Activity
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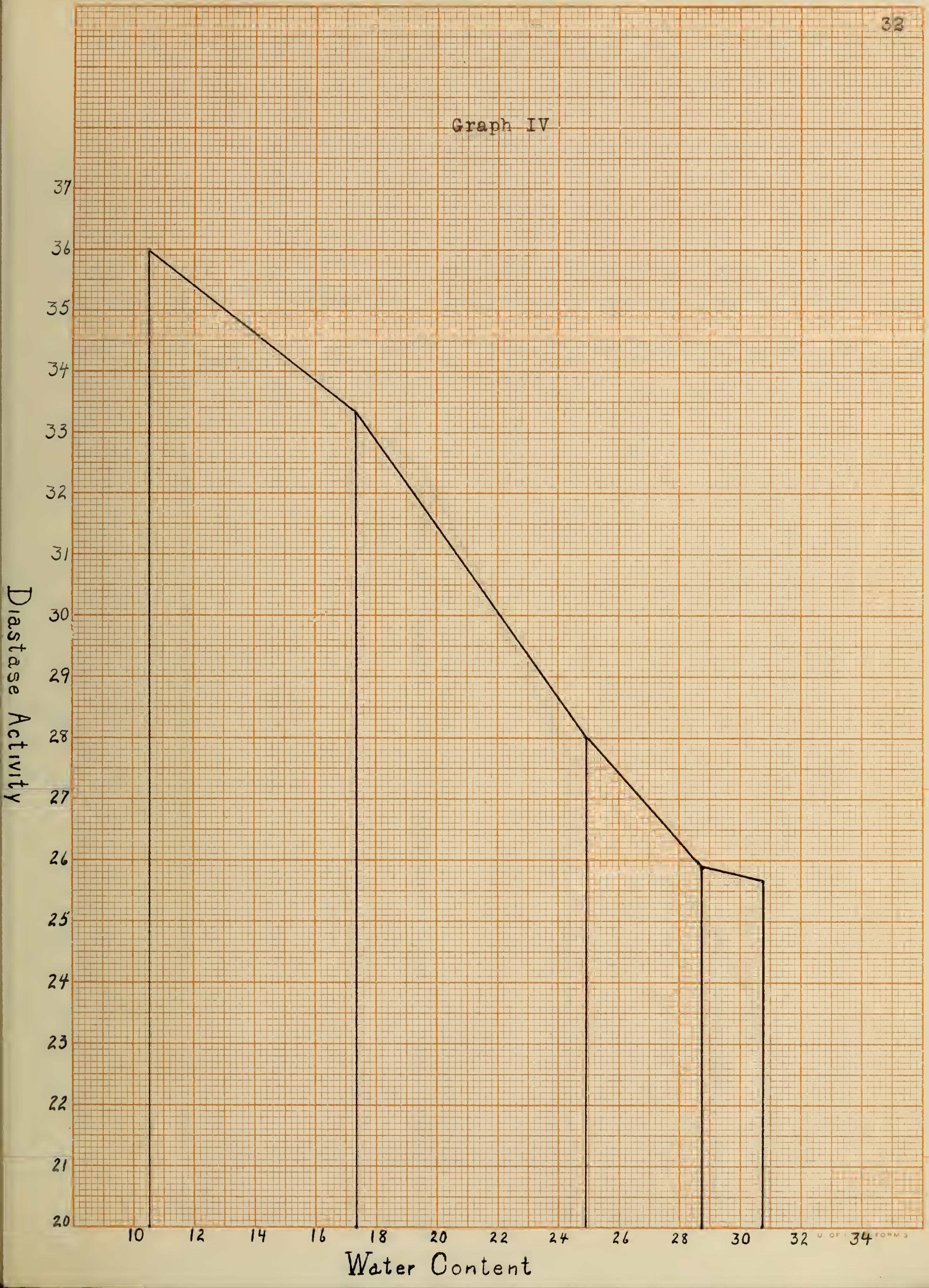
Water Content

Graph IV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Water Content


Graph V
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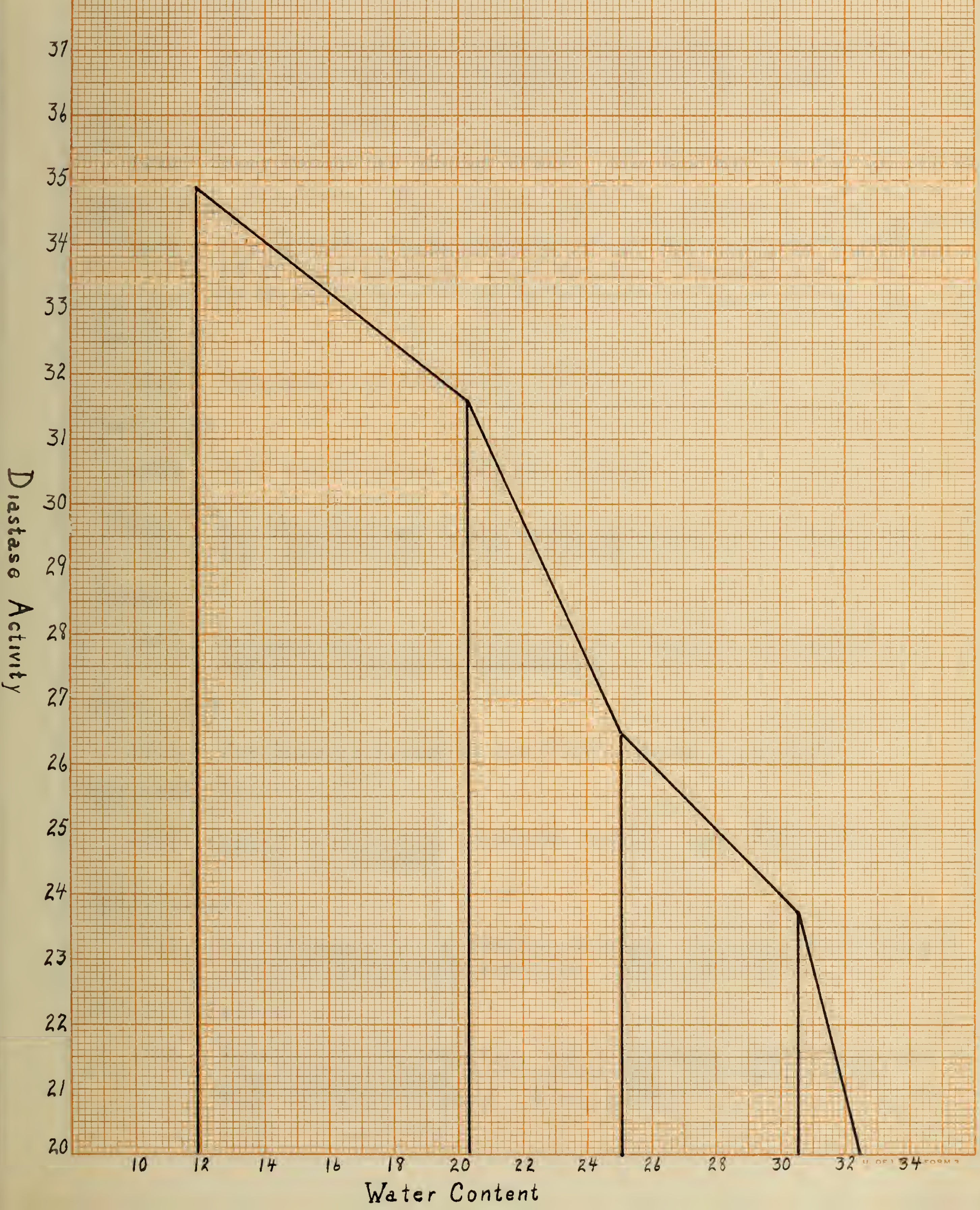
Graph VI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Water Content

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